Primer

Breaking symmetry in myxobacteria

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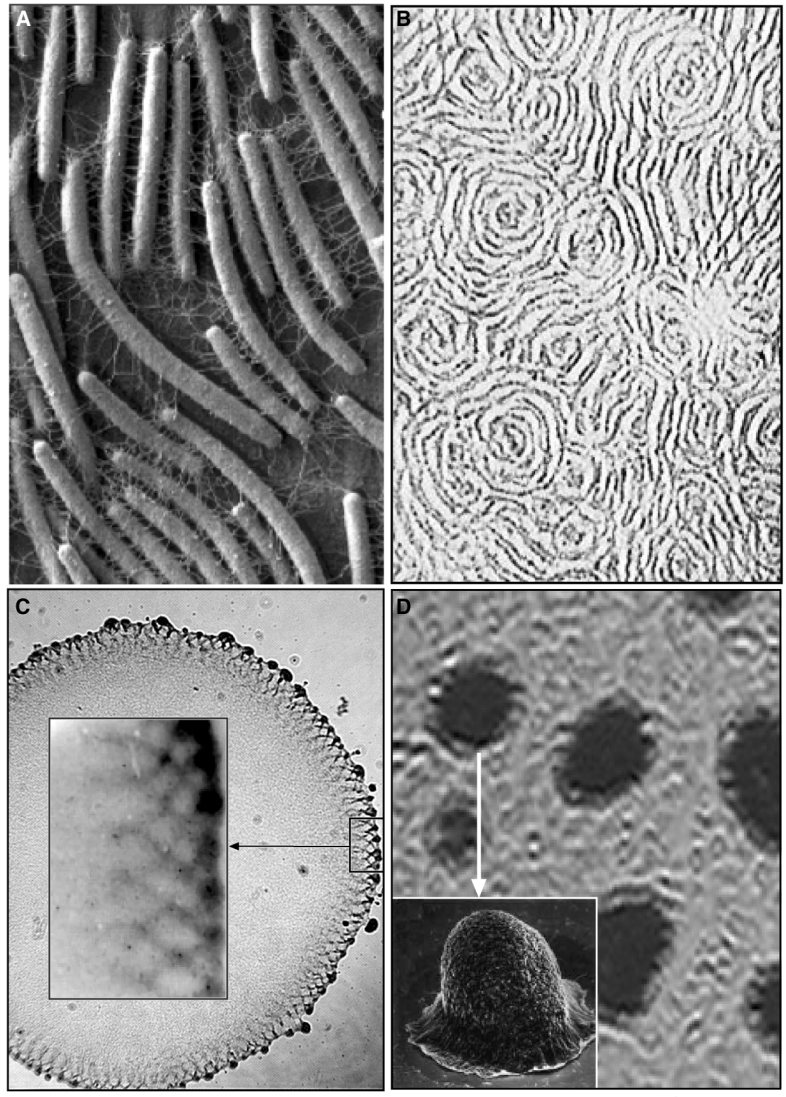
Multicellular animals and plants develop from genetically equivalent cells, yet neighboring cells often adopt different fates. Some well-studied examples are the formation of sensory bristles from a sheet of uniform neuro-ectodermal cells in *Drosophila*, and the formation of hair or feathers in the uniform skin ectoderm of vertebrates. This breaking of symmetry within groups of equivalent cells depends on cell–cell interactions that stabilize themselves. Common regulatory systems that break symmetry in flies, worms, and vertebrates use cell-surface signals such as Notch, Hedgehog and Wnt.

Although the mechanisms of cell–cell interaction during eukaryotic organogenesis have received the most attention, genetic programs capable of breaking symmetry are also found in bacteria. The view that bacteria are asocial cells with no need for intercellular communication was demolished by the discovery of homoserine lactones which carry extracellular signals and by the phenomenon of quorum sensing. In this Primer, we shall discuss the amazing patterns that arise in colonies of myxobacteria as a consequence of contact-mediated cell interactions.

Myxobacteria are common inhabitants of the soil where they enjoy a rich social life. In behavior and development, they resemble the cellular slime molds and, in some aspects of development, animals and plants as well. Myxobacteria prey on other bacteria: feeding cooperatively, they secrete enzymes that digest their prey. They compete with other soil micro-predators and, when their prey are exhausted, they stop hunting, build multicellular fruiting bodies and sporulate for survival (Figure 1C,D). This developmental program uses two cell–cell signals: first, the diffusible, quorum sensing A-signal that initiates fruiting body construction; and second, the cell surface bound C-signal that coordinates the motion of individual cells by cell-contact. We shall see some striking differences between the contact signaling system of myxobacteria and the slime molds that communicate by diffusible morphogens to pattern their fruiting bodies.

Coordinating cell motion

Myxobacteria are 5–7 μm long and about 0.5 μm in diameter, and they



Current Bioiogy

Figure 1. Myxobacteria exhibit multicellular development.
(A) Myxobacteria are cylindrical and flexible. The fibrils connecting them in this photograph provide attachment sites for Type IV pili, essential for pilus pulling and retraction during S-motility. (B) Planar, concentric, and spiral waves in a culture of myxobacteria with prominent waves. (C) A culture showing the pattern of interpenetrating waves that propagate around its periphery. The black aggregates along the boundary are nascent fruiting bodies spaced about one wavelength apart. The inset shows a detail of the counter-propagating waves and aggregates at the intersections between two waves and a band of high cell density at the edge of the culture. The arrow points to one of the aggregates. (D) Phase contrast image of the aggregates that will develop into fruiting bodies following the ripple phase. The inset is a scanning electron micrograph of a fruiting body. Movies of the waves and aggregations can be downloaded from the web site given in the references.

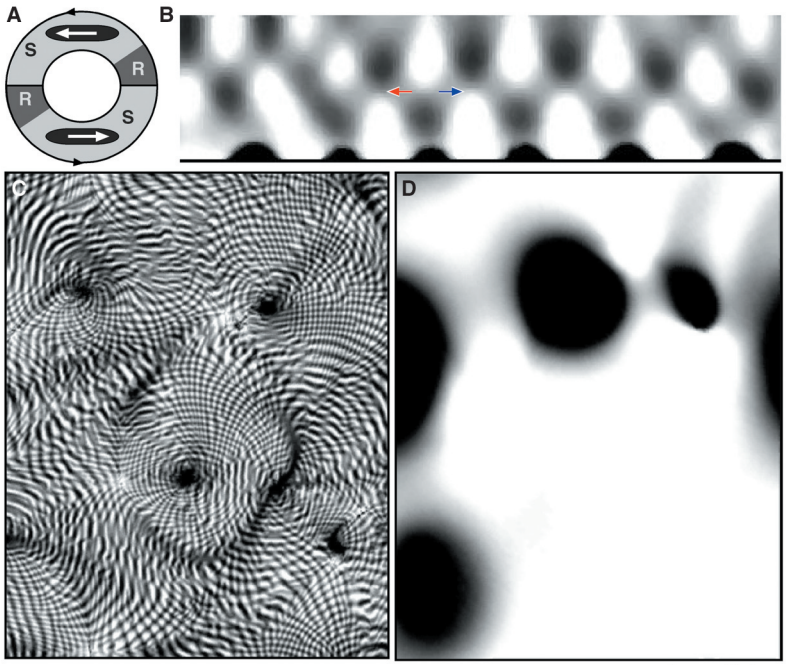


Figure 2. The mathematical model of aggregation patterns and its main results.
(A) Schematic of the reversal 'clock' controlling cell polarity. Cells moving to the right are speeded up by collisions with left-moving cells during their sensitive period (labeled S). When cells reverse, they are refractory to C-signalling for a time (labeled R). (B) Simulation of a piece of the colony boundary shown in Figure 1C. Interpenetrating waves move to the right and left (arrows), so that the wave intersections move vertically downwards depositing a higher density of cells on the colony boundary where they seed traffic jams that grow into equally spaced fruiting bodies. (C) Simulation of a two-dimensional colony with interpenetrating concentric and spiral waves similar to those shown in Figure 1B. (D) Simulation of the swirling aggregation of cells following the ripple phase when collisions suppress rather than enhance reversals, similar to Figure 1D. Movies of the waves and aggregations can be downloaded from the web site given in the references.

can bend (Figure 1A). Unable to swim, they glide in the direction of their long axis on a surface using two different motors: a pulling motor at the leading pole of the cell, and a pushing motor at the trailing pole. Fibrils serve as anchors for their pulling motors: retracting type IV pili are evident in Figure 1A as a web of thin strands that connect adjacent cells. Even though the cells are flexible, they rarely make U-turns; instead, they simply reverse their direction by trading head motors for tail motors. Contact-mediated C-signals regulate movement by altering the probability of a cell reversing direction.

Even isolated cells do not reverse at random, for their reversal times do not follow a Poisson distribution. Preceding the construction of a fruiting body a culture frequently — but not always — passes through a phase when all of the bacteria undergo fairly synchronized periodic reversals. The synchronization manifests itself in the formation of the traveling density waves: heaps of cells travel as wave crests, with cells in each heap oriented along their long axes in the direction of wave propagation. The high cell density crests visible in Figure 1B, C are separated from each other by troughs of lower cell density.

Remarkably, counter-propagating wave crests appear to pass through one another, because the unique shape of an advancing wave front is preserved after the collision. But the colliding waves only appear to interpenetrate: actually they reflect from each other. Reflection takes place at the level of individual cells that exchange C-signal when they collide end-to-end, and then respond to the signal by reversing their gliding direction. This type of wave differs from the developmental waves of the cellular slime mold *Dictyostelium discoideum* that are generated by diffusible morphogens and annihilate upon collision.

Without chemotaxis, myxobacteria aggregate by forming 'traffic jams' of thousands of cells that nucleate their fruiting bodies. Traffic jams form in regions of unusually high cell density where cells collide and are forced to slow down. The traffic jams can form with or without traveling waves, but when they accompany waves, the jams always form at points where two crests intersect the boundary of the culture, as shown in the inset to Figure 1C. Jammed cells remain motionless — stuck in traffic — for several hours.

Meanwhile, as the number of C-signal molecules rises on surfaces of cells in lower density regions adjacent to a jam, those cells begin to stream. Streaming cells move in the same direction for longer periods than they did in waves; their reversal frequency is lower and their speed of gliding is higher. Streaming cells that hit a jammed aggregate are trapped as they spiral in orbits about a traffic jam. These orbits feed and enlarge the aggregate; eventually it grows to the size of a mature fruiting body (Figure 1C,D). Why C-signal rises and how the reversal frequency changes from waves to streaming is described by a model discussed below.

Both waves and streams are organized by cell-contact-mediated C-signaling. Mutants that cannot make the C-signal protein do not change their reversal frequency during development and are defective in fruiting body morphogenesis. Both *fruA* signal transduction mutants, and *frz* mutants that fail to change reversal frequencies are also defective in aggregation; *frz* genes are homologs of the *che* genes that encode components of the chemosensory signal transduction pathway that reverses the direction of flagellar rotation in *Escherichia coli* and *Salmonella*. In *M. xanthus*, Frz signals the reversal of cell polarity.

A mathematical model

A mathematical model explains the behavior of myxobacteria in waves and swirling aggregates. The model is based on the existence of an internal biochemical cycle that controls the directional reversal cycle and the assumption this cycle is reset by reception of the C-signal (for which there is good experimental evidence). When cells in two wave crests collide, the counter-migrating crest cells transmit C-signal to each other, and the cells respond by reversing their gliding direction. Immediately after this signal-induced reversal, the cell enters a temporary refractory state in which it does not respond to C-signal. This refractory period is necessary to synchronize the phase of the reversal clocks in the colliding cells, and synchronization is the force that breaks spatial symmetry and generates patterns.

Figure 2A shows how the progression of cell-states succeed one another in a population of aligned cells. The computer simulations shown in Figure 2B show that the model successfully reproduces the patterns of counter-propagating planar waves in pre-organized populations of aligned cells like those in Figure 1C. The model also reproduces (Figure 2C) the complex patterns of spiral, concentric and planar waves shown in Figure 1B in populations of poorly aligned cells.

As they glide, the long, thin, rod-shaped cells bend and turn after head-to-side collisions with other cells, causing them to align with neighboring cells. They also tend to align because myxobacterial cells tend to turn and follow slime trails left by other cells, so that the slime trails create a kind of 'orientation memory'. Both effects enforce the local alignment of cells that gives rise to concentric, spiral, and radial wave patterns.

Cells form traffic jams when they try to glide through a region of the culture sufficiently dense to stall both gliding engines. In order to extend the model to the formation of aggregations and their subsequent growth, a stopped-state must be added to the model to take into account

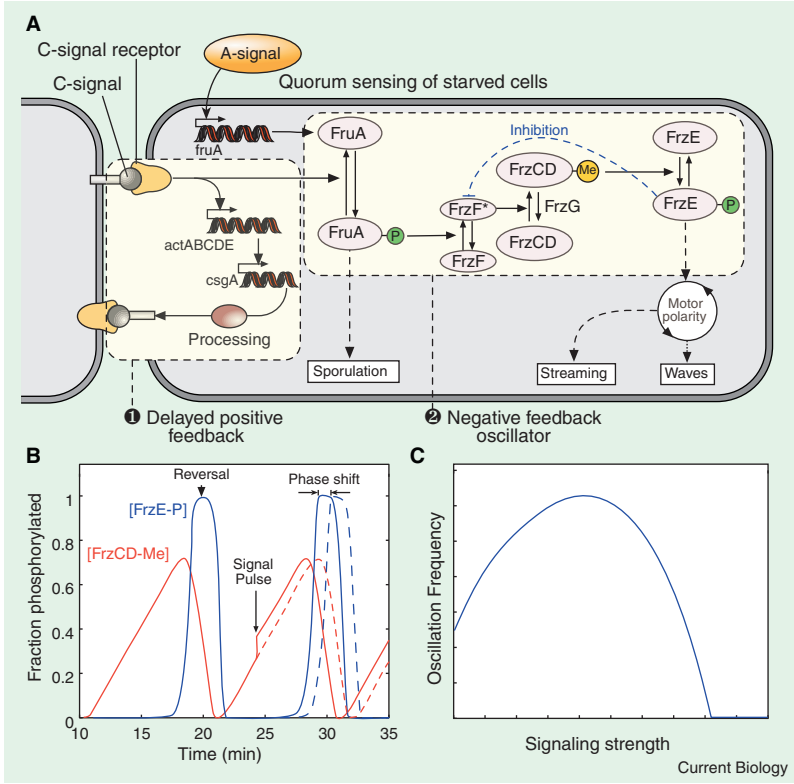


Figure 3. The reversal-regulating system of *M. xanthus*.
(A) Signaling circuit of a myxobacterial cell. The delayed postitive feedback circuit 1 is shown by the dashed box on the left. The protein products of the *act* operon respond to the C-signal by activating transcription of *csgA*, which encodes the C-signal. This increases the number of C-signal molecules on the surface of both cells. The negative feedback oscillator 2 comprises that portion of the C-signal response circuit enclosed by the dashed box on the right. Reversals, indicated in the diagram by 'Motor polarity', are induced by the phosphorylated form of the FrzE protein, an analog of CheY in *E. coli*. The phosphorylation of FrzE is induced by the methylated form of FrzCD, a homolog of the methyl accepting chemotaxis proteins, or MCP, that is cytoplasmic in *M. xanthus*. C-signaling induces the methylation of FrzCD via phosphorylation of FruA which activates the methyltransferase FrzF. FrzF* denotes the active form of FrzF. A hypothetical negative feedback loop that transforms this circuit into an oscillator is shown by a blue dashed line. The phosphorylated form of FrzE inhibits methylation of FrzCD by deactivating FrzF. (B) Simulation of the Michaelis-Menton kinetics of the signaling circuit. Oscillation of the negative feedback circuit produces a gradual rise of the activated fraction of FrzF. This induces methylation of FrzCD and subsequent phosphorylation of FrzE. The latter induces a reversal and feeds back to reduce the amount of activated FrzF. A pulse of C-signaling increases the activated FrzF fraction and results in a shift of the FrzE maximum leftward leading to a faster reversal. During the falling phase of the FrzCD-Me cycle the system is refractory to C-signaling. (C) The oscillation frequency first increases with signaling strength (FrzF activation rate), but eventually falls, and oscillations cease above some critical value of that strength. This reproduces the developmental progression of the colony from waves through swirling and aggregation.

these traffic jams that form at high cell densities. In cultures like that shown in Figure 1B, high cell densities are found at the triple intersection points of two colliding wave crests and the band of high cell density at the edge of the culture. The aggregates that initiate at these intersections are equally spaced about a wavelength apart. The traffic jams are too dense to be penetrated by cells that approach them, and so cells are deflected aside and glide in spiral trajectories around the traffic jams. This produces a circumferential orientation around each jam and transforms it from an asymmetric structure to one with radial symmetry. Because of a positive feedback in the circuit the cells' responses to C-signaling, the number of C-signal molecules per cell at this stage has increased (see Figure 3 and the discussion below). Now a cell that catches up

and collides with a cell that is moving ahead of it continues moving in the same direction. Reversals of both cells are suppressed and they segregate as a unidirectional stream moving toward an aggregate. Because they cannot penetrate the aggregate, the stream is deflected and orbits around the aggregate. Streams also bring about the exchange of cells between adjacent circulating aggregates and the two eventually fuse with each other; such fusions are frequently observed in fruiting cultures. The mathematical model predicts the swirling aggregation patterns (Figure 2D) like those observed experimentally (Figure 1D).

Where is the reversal clock?

Figure 3A summarizes the known components of the reversal regulating system in *M. xanthus*. The scheme incorporates current biochemical and mutational studies as well as known sequence homologies with the well characterized che system of *E. coli*. There are two essential features of this system. First, there is a positive feedback loop that results in accumulation of more C-signaling protein on the cell surface following each C-signaling event. Second, the C-signal is processed through a cascade of covalent modifications that ultimately control reversals in the cell polarity. This cascade can be easily transformed into a biochemical oscillator if a negative feedback loop is introduced. For instance, the phosphorylated form of FrzE can inhibit activation of the methlyltranferase FrzF (this feedback is shown in Figure 3A by the dashed blue line).

Similar results can be achieved if the phosphorylated form of FrzE activates the demethelylase FrzG instead. The period of the resulting oscillator in the absence of signaling can be tuned to the 8–10 minute reversal period of isolated cells. A cell collision produces a pulse of C-signaling that induces a phase shift that speeds up cell reversal, as illustrated in the computation shown in Figure 3B. Moreover, prolonged exposure to C-signaling first causes the oscillation frequency to increase, as found in the ripple phase. Eventually, however, the deactivation of FrzF becomes rate limiting as more and more FruA is phosphorylated, and the reversal frequency decreases leading to the streaming phase. Ultimately, all of the FruA is phosphorylated so that FrzF is permanently active and the oscillation ceases. This developmental sequence is predicted by the model as plotted in Figure 3C.

The computed properties of the oscillator are consistent with the observed behavior of myxobacterial cells. During the ripple phase cells receive pulses of C-signaling at each collision and reverse faster. Eventually, the cells accumulate significant amounts of C-signal on their surfaces, which begins to inhibit their reversals and the cells enter their streaming phase. The oscillator model is also consistent with the existence of the refractory period and cooperativity of signaling, both of which are essential for generating the unique properties of the density waves. A detailed description of the 'Frzilator' (Frz-oscillator) will be published elsewhere.

A similar circuit in eukaryotic development?

The interpenetrating waves of myxobacteria are unlike those described thus far in chemistry or biology. Their formation depends on the cells' periodic reversals of polarity and their synchronization by contact mediated signaling. This has intriguing similarities to the 'clock-wavefront' model for somitogenesis in vertebrates. Somites are transient periodic structures that form along the embryonic axis and presage the vertebral segments. They assemble from pre-somitic mesoderm following a kinematic wave that progresses in an anterior-to-posterior sequence. The randomly organized mesoderm is converted at the front of the wave to a repeating array of oriented epithelial structures. To synchronize the conversion, an internal negative feedback oscillator is proposed.

One recent model for this process resembles the myxobacteria orientation clock: a negative feedback oscillator resulting from transcription delays is installed in each cell. Cells synchronize with their neighbors by direct contact via the Delta–Notch signaling system (Lewis, 2003). Somite morphogenesis involves the reorganization of mesoderm into orientated epithelia. Just as slime mold morphogenesis has been used as a metaphor for metazoan morphogenesis by diffusible morphogens, myxobacteria may be a useful metaphor for metazoan pattern formation that is mediated by cell contact.

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Further reading

Igoshin, O.A., Welch, R., Kaiser, D. and Oster, G. (2004). Waves and aggregation patterns in Myxobacteria. Proc. Natl. Acad. Sci. USA *101*, 4256-4261.

Igoshin, O.A., Mogilner, A., Welch, R.D., Kaiser, D. and Oster, G. (2001). Pattern formation and traveling waves in myxobacteria: Theory and modeling. Proc. Natl. Acad. Sci. USA *98*, 14913-14918.

Kaiser, D. (2003). Coupling cell movement multicellular development in myxobacteria. Nat. Rev. Microbiol. *1*, 46-54.

Lewis, J. (2003). Autoinhibition with transcriptional delay: A simple mechanism for the zebrafish somitogenesis oscillator. Curr. Biol. *13*, 1398-1408.

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